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БИОЛОГИЧЕСКИЕ И БИОХИМИЧЕСКИЕ МЕТОДЫ АНАЛИЗА И КОНТРОЛЬ СОСТОЯНИЯ ОКРУЖАЮЩЕЙ СРЕДЫ (METHODS IN THE BIOLOGICAL AND BIOCHEMICAL SCIENCES FOR ENVIRONMENTAL MONITORING)

В работе рассмотрены методы экологического мониторинга, которые могут быть классифицированы на биологические и биохимические. Показано, что отрицательные биологические эффекты загрязняющих веществ, присутствующих во всех видах проб окружающей среды, могут быть оценены с использованием различных живых организмов или клеток в качестве «аналитических приборов». Названы преимущества широкого использования микроорганизмов для различных биопроб из-за легкости и низкой стоимости их культивирования, а также отсутствия этических проблем, часто сопровождающих использование высших организмов.

In the past few decades, environmental pollution has become one of the world's major concerns. A great number of toxic compounds, originating mostly from industrial and agricultural activities, are being released in to our environment continuously. In some cases harmful chemicals induce strong acute toxic effects to exposed organisms when released to the environment, but frequently the consequences are delayed due to the effects of bioaccumulation and biomagnification. Early detection of toxic chemical compounds in the environment, particularly in water, and their biological effects on organisms has therefore become increasingly important.

The traditional approach to environmental pollution assessment is based on chemical analytical methods which only provide information about the absolute concentrations of known chemicals in the environmental sample without an adequate interpretation of its toxicity to biota in the context of bioavailability, which means it only provides information about their potential, not actual toxicity. Moreover, compounds that are toxic below the detection limit of chemical

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analytical method or new compounds that are not yet deposited in the databases cannot be detected this way. Another disadvantage of chemical methods is the lack of information about the combined toxicity of different compounds such as additive, synergistic or antagonistic effects. In order to get more relevant information about environmental pollution risk, it is therefore inevitable to supplement the chemical analytical data with the results of methods providing information on biological impacts.

The negative biological effects of pollutants present in all kinds of environmental samples can be assessed using different living organisms or cells as 'analytical devices'. The biological response following the exposure of living organisms or cells to environmental sample usually gives an information on toxicity, genotoxicity, estrogenicity etc. of the whole mixture of chemical compounds present in that particular sample. Besides being sensitive only to the bioavailable fraction of pollutants, biotests also have the power to assess the integrated effect of interacting chemical compounds and to detect the compounds, which are toxic only due to bioactivation.

According to the technical principle, methods of environmental monitoring can be classified to biological and biochemical.

The article «The applications of microbes in environmental monitoring» of R. Marinšek Logar and M. Vodovnik is given several methods of analysis [1]. For example, bioassay or ecotoxicity assay is an experiment in which living test-species are exposed directly to an environmental sample (soil, sediment, surface water, ground water, waste water..) or extract of an environmental sample to measure a potential biological effect due to the presence of potential contaminants. Microbial bioassays can roughly be divided to (general) toxicity assays and genotoxicity assays. The purpose of ecotoxicity bioassays is to assess the integral effect of an environmental sample on general physiological state of the test-species, while genotoxicity tests specifically show the effects resulting in changes of genetic material. Many of them are also standardized and commercially available. Another method is to microbial biosensors. A biosensor is defined as a self-contained, integrated device, consisting of a biological recognition element interfaced to a physical signal transducer, that together reversibly respond to a chemical species in a concentration-dependant manner. A wider definition also includes some other forms of biological sensors, including genetically engineered microorganisms, which respond in observable ways to target analyte or group of related analytes. Microbial biosensors for environmental applications range in their development stages from proof-of concept to full commercial availability. Regarding the target detection specifity they may fall in one of two groups. You also need to talk about immunoassays. Immunochemical methods are based on specific and reversible binding of immunoglobulin molecules (antibodies) to their target antigenes. The most popular immunochemical technique in environmental analyses today is immunoassay, which has been

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shown to detect and quantify many compounds of environmental interest such as pesticides, industrial chemicals, and products of xenobiotic metabolism.

The use of biological methods in environmental monitoring is essential in order to complement chemical analysis with information about actual toxicity or genotoxicity of environmental samples. Microorganisms are widely applied test-species in different bioassays because of the ease and low costs of their culturing as well as the lack of ethical issues often accompanying the use of higher organisms. Combining biology to engineering skills has enabled the development of biosensors - new generation of analytical devices coupling biological recognition elements to physical signal transducers. Besides the direct application of whole microorganisms or their isolated parts for general toxicity assessment or detection of specific compounds, genetically modified microbes also represent an important source of recombinant antibody production, which makes them important also when talking about immunoassays.

Unlike the previous article, which covers the entire environment in article «Biological Monitoring» prepared by D. Chapman and J. Jackson [2] with contributions from F. Krebs much attention is given to aquatic ecosystem.

One of the primary methods is ecological method. Each aquatic organism has particular requirements with respect to the physical, chemical and biological condition of its habitat. Changes in these conditions can result in reduction in species numbers, a change in species dominance or total loss of sensitive species by death or migration. The presence or absence of certain species in relation to particular water quality characteristics has been exploited in the development of ecological methods based on “indicator species”. These methods are frequently referred to as biotic indices and require a good knowledge of the organisms in the specific environments to which the methods are applied. Information on the physical and chemical status of the aquatic habitats in which these methods are used is also essential in order to determine whether certain species could survive there, even under undisturbed conditions. Equally important it is physiological technique. For the purposes of water quality monitoring, the most widely exploited physiological responses of aquatic organisms to environmental stress are production, respiration and growth rates. Most of these responses have been developed for biological monitoring under controlled conditions, such as during bioassays. The growth criteria (light, nutrients, temperature) for some common freshwater algal species have been well studied and documented and several methods based on algal growth rates have now been standardized. For most physiological methods the results can only be considered as relative. Nevertheless, such methods are useful for monitoring large areas, along long river stretches, or for short, intensive programmes. In addition, some methods are particularly useful for monitoring the effects of effluents, where measurements can be made upstream and downstream of the discharge. Also we cannot do without controlled biotests. Bioassay methods can be used to reveal or confirm

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the presence of toxic conditions in water bodies as well as to provide information on the toxicity of effluents. Bioassay methods can be used to demonstrate the presence of “unknown” contaminants, to locate the position of diffuse or point discharges of contaminants or to monitor the dispersion of known toxic discharges. In addition, such methods are useful for evaluating persistence and the combined effects of several contaminants or effluents.

Many biological approaches can be cheaper than chemical methods in terms of equipment, but would normally place heavy demands on field and laboratory personnel. Financial savings can sometimes be made in a monitoring programme by using biological methods to “trigger” the need for intensive and sensitive chemical analyses.

A disadvantage of biological methods is that it can be difficult to relate observed effects to specific aspects of environmental disturbance, such as contamination or natural changes. For example, methods do not always provide precise information on the identity of a contaminant unless supplementary information from chemical analyses is available. In addition, the response of organisms may be affected by their natural cycles, such as life stage and reproductive condition. Consequently, like other techniques, biological monitoring methods should be developed and interpreted by experienced specialists.

Not only ordinary microorganisms, plants and animals used in biological and biochemical analysis methods. For example, in the article «Bioindicators in environmental monitoring: bioluminescent bacteria, algae and honeybees» already from the name it is clear thet use bioluminescent organisms [3].

The luminescence based bioassays for the ecotoxicological assessment of environmental pollutants have been used since several decades ago. Bioluminescent organisms, naturally or genetically modified to display this feature, free or immobilized to prepare a biosensor probe, can offer the possibility to perform rapid, sensitive, reproducible and cost-effective assays for toxicity screening and assessment in water, sediments, and soils, also offering the further advantage of an easy record of the effects produced on the living organism light emission.

The bioluminescent bacteria (BLB) tests protocol is usually simple, especially when applied to aqueous samples or extracts: the bacteria emit light when they find themselves in optimal conditions whereas in presence of noxious substances their luminescence decrease. Thus the presence of toxic molecules, as pesticides, heavy metals, organic and inorganic compounds, can be evaluated.

Microalgae are the primary producers at the base of the aquatic food chain. They are one of the first groups to be affected by contamination and therefore they provide important information for predicting the environmental impact of pollution in water bodies. Algal tests are generally sensitive, rapid and cost effective and are based on the measurement of physiological (growth and photochemistry) or biochemical (enzyme activity, oxidative stress response) changes as test end points.

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Honeybees have been identified as a good biological indicator since the beginning of last century, because they detect and reveal the chemical impairment of the environment they live in, mainly through two signals. One is the most evident, i.e. the changes in the mortality, while the second one is represented by the residues collected from the environment on or within their bodies or in beehive products and that may be evaluated by suitable analyses.

The various biosensors used during researches demonstrated their suitability and sensitivity in detecting the different pollutants. Nevertheless, it is acknowledged that the "battery of test" approach, utilizing several different short-term biological tests, would be preferred in any monitoring scheme, since the specific or unspecific sensitivity of an organisms to pollutants represents just its answer to it, not all the possible effects on the whole biosphere.

Due to a wide variety of chemical compounds, the problem of toxicity of polluting substances is difficult to define. However, appropriate interpretation of research results and environmental changes allow us to assess environmental pollution by biological and biochemical methods. The development of analytical chemistry makes it possible to use new, more effective monitoring techniques, determining the effects of bad ecology on humans and animals. To obtain a complete and reliable picture of the ecosystem, it is necessary to compare information provided by particular bioindicators. Environmental pollution constitutes a serious threat, so biomonitoring methods should be constantly improved, to enable prediction and control of potential environmental hazards. Nowadays it is a well-known fact that each ecosystem component can provide valuable information about degradation of the natural environment and dangers to human and animal health resulting from it. Beyond a doubt, acquiring knowledge about ecological tolerance and its application to practice can be of benefit to us all.

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